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Functionalized *C*-Glycoside Ketohydrazones: Carbohydrate Derivatives that Retain the Ring Integrity of the Terminal Reducing Sugar

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Glycosylation often mediates important biological processes through the interaction of carbohydrates with complementary proteins. Most chemical tools for the functional analysis of glycans are highly dependent upon various linkage chemistries that involve the reducing terminus of carbohydrates. However, because of ring opening, the structural integrity of the reducing sugar ring (pyranose or furanose) is lost during these techniques, resulting in derivatized carboydrates that markedly differ from the parent molecule. This paper describes a new aqueous-based, one-pot strategy that involves first converting the sugar to a *C*-glycoside ketone, followed by conversion to ketohydrazones or oximes. Hence, the *C*-glycoside ketones are tagged with fluorescence, colored, cationic or biotin-labeled groups or immobilized onto hydrazine-functionalized beads. No activating or protecting groups are required, and the chemistry is mild enough for a wide range of carbohydrates. We demonstrate the versatility of the approach to diverse glycans, including bead immobilization and lectin analysis of acarbose, an antidiabetic drug, to dabsyl-tagged enzyme substrates to screen cellulases, and for the analysis of plant cell wall hemicellulosics.

Genomic and proteomic techniques are widely used to elucidate the role of genes and proteins in biological systems. Until now, the corresponding techniques to explore the role of carbohydrates in biology, the growing discipline of glycomics, has tended to lag behind. Carbohydrates have long been known to be important in a variety of cellular process, including inflammation, signal transduction, fertilization, development, and cell−cell, bacterium−cell, and virus−cell recognition.[1−5] A number of glycomics tools are becoming available, including fluorescent and colorimetric glycoconjugates, sugar microarrays, quantum-dot conjugates, affinity-tagged carbohydrates, derivatized microspheres, and affinity resins.[6−10] However, rapid advances have been hindered by the structural complexity and diversity of monosaccharides, glycoconjugates, oligosaccharides, and polysaccharides, and the relative difficulty of isolating, characterizing, and synthesizing such complex heterologous structures.

Most glycomics tools are highly dependent upon various linkage chemistries that involve the reducing-terminus of carbohydrates.[11−17] Numerous techniques for the derivatization of sugars have been described, including reductive amination and the formation of reducing sugar hydrazones, thiazolidines, and oximes (Figure 1). However, because of ring-opening, the structural integrity of the reducing sugar ring (pyranose or furanose) is not retained during these techniques, which thus results in derivatized oligosaccharides that markedly differ from the parent molecule. Reductive amination methods are based on imine formation between an amine reagent and the anomeric carbonyl group of a reducing carbohydrate, followed by reduction to an open-chain glycamine derivative. The imines themselves are generally unstable, with the equilibrium favoring free carbonyl, except when stabilized by alpha-nitrogens such as in hydrazones and oximes.[18] Hydrazones and oximes are excellent derivatives for the analysis of simple aldehydes and ketones, but sugars do not usually form simple hydrazones. Rather, they react with three equivalents of aryl- or acyl-hydrazines at both the C-1 and C-2 carbons to give sugar osazones. An imine initially forms at the anomeric C-1 carbon. The adjacent hydroxyl is then oxidized to a carbonyl group, which reacts with a third equivalent of the

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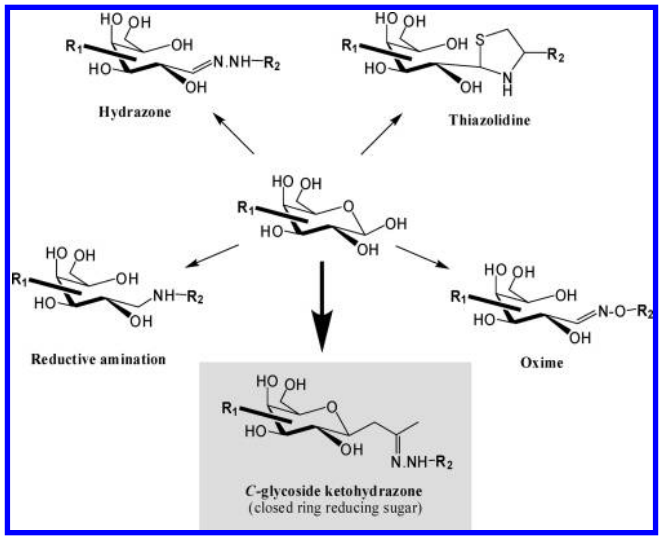

Figure 1. Approaches to carbohydrate end-labeling. The general problem of sugar ring-opening encountered with hydrazone, thiazolidine, or oxime formation or with reductive amination. *C*-Glycoside ketohydrazones alleviate this problem by retaining the ring integrity of the terminal reducing sugar.

hydrazone to give an osazone. Because the reaction is at C-1 and C-2, aldoses and related ketoses (glucose and fructose, for example) give the same osazone, as do 2-epimer sugars such as mannose and glucose. Furthermore, as with reductive amination, oximes, and thiazolidines, the derivatized residue is acyclic and retains little of the structural characteristics of the original sugar.

The need, therefore, remains for an improved process for preparing sugar derivatives wherein the ring structure of the reducing sugar is not opened. This paper describes new carbohydrate-based derivatives, *C*-glycosidic ketones, and the subsequent formation of various functionalized ketoximes and ketohydrazones (phenyl, dansyl, dabsyl, Girard's P reagent, biotinyl). Mono- and oligosaccharides give high yields from aqueous-based, one-pot reactions, and the functionalized *C*-glycosides retain the closed ring conformation of the parent sugar. No protecting group manipulations or activation of the anomeric center was required. This strategy is suitable for the derivatization of small quantities of naturally occurring carbohydrates and so has high potential for end-labeling and immobilization of carbohydrates for various applications in the field of glycomics.

EXPERIMENTAL SECTION

Source of Carbohydrates and Chemicals. Malto- and cellulo-oligosaccharides, and acarbose were obtained from Sigma-Aldrich, St. Louis, MO. Gentio-oligosaccharides were from Suntory Corporation, Osaka, Japan, and xylo-oligosaccharides were from Waco Pure Chemicals, Osaka, Japan. Chitotetraose was obtained from Seikagaku America, East Falmouth, MA, and the $Man_8GlcNAc_2$ *N*-glycan was from Glycotech, Gaithersburg, MD. Other reagents, including derivatization and matrix chemicals, and solvents for MALDI-TOF MS, were of the highest obtainable purity available.

Synthetic Methods. Carbohydrate derivatives were prepared by an aqueous-based one-pot reaction, essentially as described.[19−21] The *C*-glycoside ketones were prepared from the corresponding unprotected sugars (oligosaccharides or monosaccharides) by reaction with a β-diketone. The sugars were reacted on a milligram scale in bicarbonate buffer (aq. $NaHCO_3$; 38 $g \cdot L^{-1}$; pH 8.5) with an approximate 1.1 molar excess of the diketone. After 4 h at 80 °C, the reactions were cooled, neutralized with Dowex 50 W resin and washed by extraction with ethyl acetate. The completeness of reaction was assessed by MALDI-TOF MS. For 2-deoxy-2-acetylamino sugars, optimum yields were obtained after further reaction with a second aliquot of diketone. The *C*-glycoside ketone solutions were converted to their ketohydrazones or oximes by shaking (1 h, room temperature) with a slight molar excess of the appropriate acylhydrazide or hydroxylamine reagents. For the water insoluble hydrazide reagents (e.g., dansyl- and dabsyl-hydrazide), quantitative yields were attained by addition of an equivalent volume of dimethylformamide.

Mass Spectrometry Analysis. MALDI-TOF mass spectra were recorded on a Bruker-Daltonic (Billerica, MA) Omniflex instrument operating in reflecton mode. Aqueous derivatization mixtures (typically 2 μL) were mixed with 2,5-dihydrobenzoic acid matrix (10 μL; saturated in acetonitrile) and cocrystallized on a conventional 49-place target. Ion source 1 was set to 19.0 kV and source 2 to 14.0 kV, with lens and reflector voltages of 9.20 and 20.00 kV, respectively. A 200 ns pulsed ion extraction was used with matrix suppression up to 150 Da. Excitation was at 337.1 nm, typically at 75% of 150 μJ maximum output, and 60 shots were accumulated. Fragmentation of the native and labeled glycans was determined using a quadrupole orthogonal time-of-flight (Q-oTOF) mass spectrometer (Applied Biosystems/MDS Sciex QSTAR Elite, Toronto, Canada) with turbospray ionization. Samples were diluted in isopropanol/water (1:9) at a final concentration of 5 pmol/μL and introduced at a flow rate of 10 μL/min. Compositional GC/MS data was obtained for the peracetylated *C*-glycoside ketones as described[21] using an Agilent 6860N/5973 instrument (Agilent, Santa Clara, CA).

Enzymatic Methods. Commercial cellulases were obtained from EMD-Calbiochem, San Diego, CA, and Cooper Biomedical, Malvern, PA. Cellobiose-*C*-glycoside and glucose-*C*-glycoside dabsyl ketohydrazones were prepared and incubated with the appropriate enzyme at 40 °C in 100 μL of 5 mM sodium acetate buffer, pH 5.0. Aliquots (10 μL) were removed after 3 h and mixed with and equal volume of 2,5-DHB matrix in acetonitrile prior to analysis by MALDI-TOF MS.

Other Methods. Acarbose-*C*-glycoside biotin ketohydrazone was prepared by treatment of acarbose-*C*-glycoside ketone with commercial biotin hydrazide. We immobilized this onto streptavidin-labeled agarose resin beads (1 μM particle size, Sigma-Aldrich, St. Louis, MO), washed with 0.1% Triton X-100 and several times with water and then incubated with fluorescent FITC-concanavalin A in 10 mM PBS, pH 7.2. Control beads lacked the acarbose-*C*-glycoside biotin ketohydrazone. Following several further washings with 0.1% Triton X-100 and water, the beads were examined by fluorescence microscopy (Zeiss SteREO Discovery

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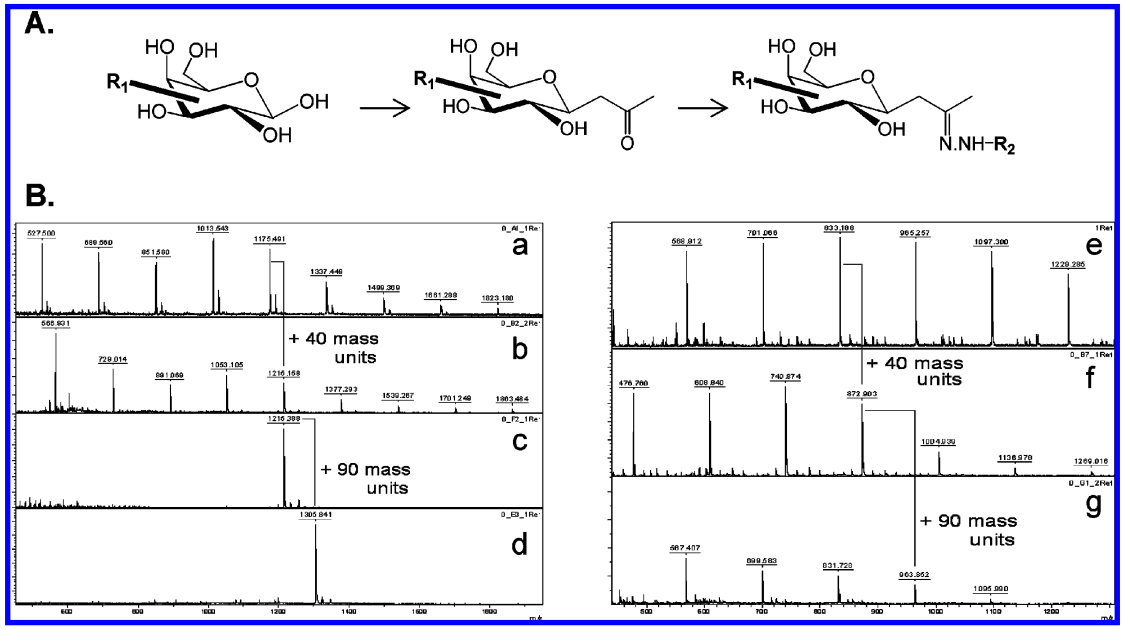

Figure 2. Oligosaccharide *C*-glycoside ketohydrazones. (A) Generalized reaction scheme. R_1 = malto-, cellulo-, xylo-, chito-, gentio-oligosaccharides, etc. R_2 = phenyl, dansyl, Girard's reagents, dabsyl, biotinyl, etc. (B) Quantitative formation of oligosaccharide *C*-glycoside ketohydrazones analyzed by MALDI-TOF MS. a, nonderivatized malto-oligosaccharides (dp 3−11); b, corresponding malto-oligosaccharide *C*-glycoside ketones (increased by 40 mass units); c, maltoheptaose *C*-glycoside ketone; d, corresponding maltoheptaose *C*-glycoside phenyl ketohydrazone (+90 mass units); e, nonderivatives xylo-oligosaccharides (dp 4−9); f, corresponding xylo-oligosaccharide *C*-glycoside ketones (+40 mass units); g, corresponding xylo-oligosaccharide *C*-glycoside phenyl ketohydrazones (+90 mass units).

V8 and Axio Imager A1 microscopes, Carl-Zeiss Microimaging, Thornwood, NY).

RESULTS

Aqueous-Based Formation of *C*-Glycoside Ketone. The formation of *C*-glycoside ketones from monosaccharides and water-soluble β-diketones has been investigated by Lubineau[19] and by Fessner et al.[20] The reaction typically involves an unprotected aldose sugar with a simple β-diketone such as pentane-2,4-dione (acetylacetone) in aqueous sodium bicarbonate and proceeds with excellent yields. We used MALDI-TOF MS as a tool to monitor the reactions with a variety of monosaccharides, with the expectation that the *C*-glycoside ketones would give rise to $[M + Na]^+$ molecular adduct ions 40 mass units larger than those for the parent sugar (Figure 2 and Supporting Information, Figure S.1.).

Following reaction with acetylacetone, all major naturally occurring monosaccharides were quantitatively converted into their *C*-glycoside ketone derivatives, as characterized by the appropriate $[M + Na]^+$ and more minor $[M + K]^+$ ions (Supporting Information, Figure S.1.). When the acetylacetone was replaced with heptane-3,5-dione in the reaction, the resulting hexose-based *C*-glycoside ketones gave rise to $[M + Na]^+$ and $[M + K]^+$ ions at m/z 257.1 and m/z 273.1, respectively. These ions are 14 mass units larger than those for the corresponding acetylacetone-derived *C*-glycoside ketones, consistent with the structure (Supporting Information, Figure S.1.). Nonderivatized monosaccharides were not detected after these reactions, thus, confirming the quantitative conversion. The fact that these spectra were obtained directly on the reaction supernatants without any prior purification or chromatography strongly supports the efficiency and cleanness of the chemistry. To confirm these monosaccharide *C*-glycoside structures, some of which have been reported previously,[19−21] the reaction products were peracetylated under Zemplen conditions and analyzed by GC/MS. Each gave rise to a single chromatographic peak consistent with the formation of a single product.[21]

Oligosaccharide-Based *C*-Glycoside Ketones. The success of the *C*-glycoside ketone chemistry with monosaccharides prompted us to extend the work to include several oligosaccharides. The use of the MALDI-MS analysis allowed for rapid monitoring and identification of product ions. Hence, the reaction of acetylacetone with maltoheptaose was observed to give rise to a single product ion at m/z 1215 for the expected $[M + Na]^+$ of the maltoheptaose *C*-glycoside ketone (Figure 2). Moreover, the reaction with pentose monosaccharides, ribose and xylose, suggested that the chemistry might also be applicable to pentose-containing oligosaccharides. Hence, xylo-oligosaccharide *C*-glycoside ketones were also produced quantitatively from a series of xylo-oligosaccharides (Figure 2). *C*-glycoside ketones were also prepared from various other neutral hexose-containing oligosaccharides (cellulo-, malto-, gentio-, lacto-) or with *N*-acetylhexosaminyl oligosaccharides (chitins and *N*-glycans) showing the methodology is broadly applicable to sugars with differences in anomeric linkage or structure (Figure 2 and Supporting Information). Moreover, as discussed below, further work established the general scope and utility with several heterologous oligosaccharides.

Functionalized *C*-Glycoside Ketohydrazones. The introduction of the ketone group at the carbohydrate reducing end immediately suggested the possibility of further derivatization with ketone-reactive reagents such as hydroxylamines and hydrazines. Reaction of the malto- and xylooligosaccharide *C*-glycoside ketones with phenylhydrazone quickly established the potential of this approach (Figure 2). Maltoheptaose *C*-glycoside ketone (dp 7) and the dp series of xylo-oligosaccharide *C*-glycoside ketones were

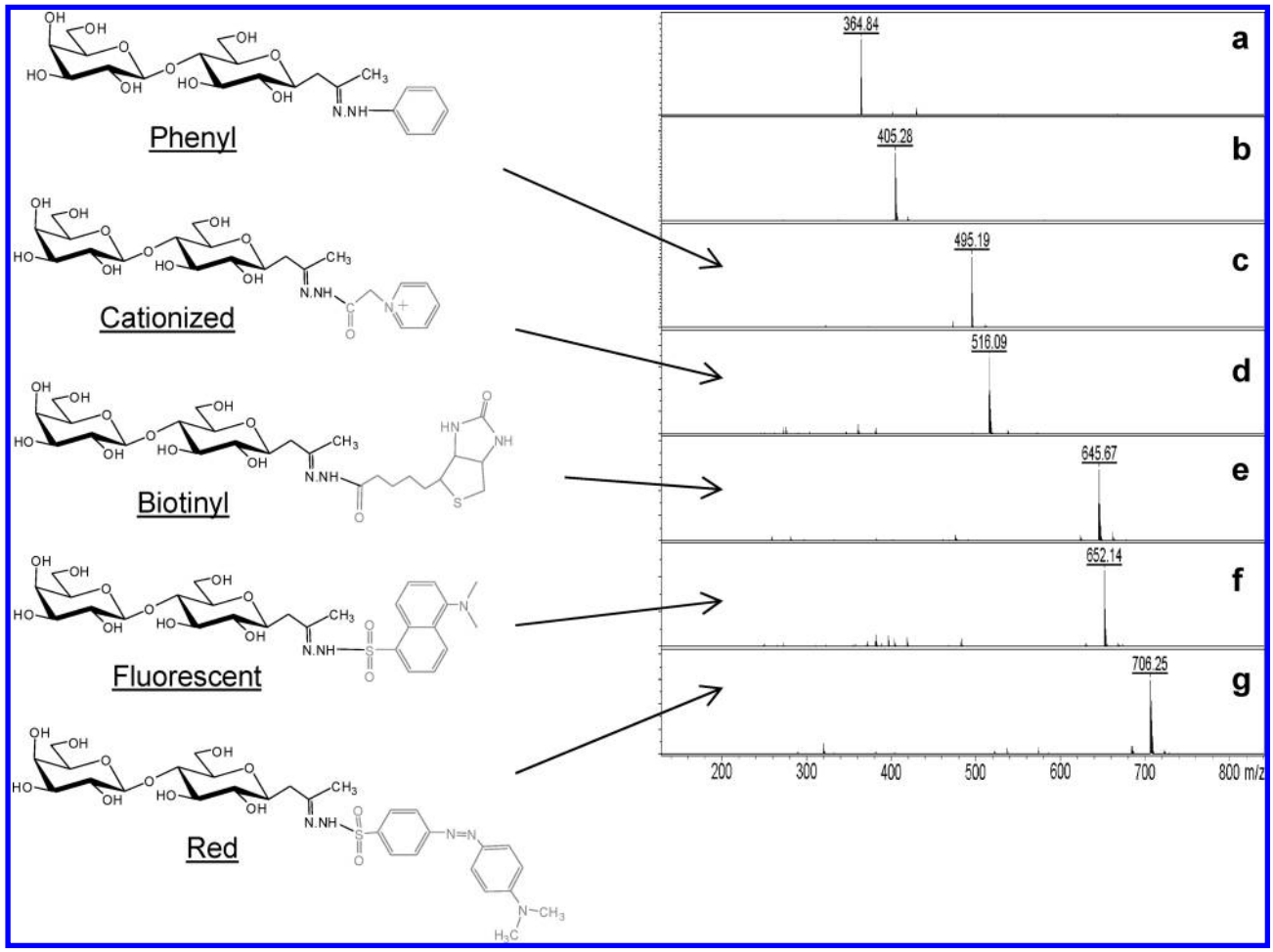

Figure 3. C-Glycoside ketohydrazone carbohydrate-labeling groups. MALDI-TOF MS spectra of a, lactose (D-Gal-β-1,4-D-Glc, $C_{12}H_{22},O_{11}$, calc. $[M + Na]^+ = 365.11$); b, lactose *C*-glycoside ketone ($C_{15}H_{26},O_{11}$, calc. $[M + Na]^+ = 405.14$); c, lactose *C*-glycoside phenyl ketohydrazone ($C_{21}H_{32},N_2O_{10}$, calc. $[M + Na]^+ = 495.19$); d, lactose *C*-glycoside pyridinium ketohydrazone ($C_{22}H_{34},N_3O_{11}$, calc. $M^+ = 516.22$); e, lactose *C*-glycoside biotinyl ketohydrazone ($C_{25}H_{42},N_4O_{12}S$, calc. $[M + Na]^+ = 645.24$); f, lactose *C*-glycoside dansyl ketohydrazone ($C_{27}H_{29},N_3O_{12}S$, calc. $[M + Na]^+ = 652.21$); g, lactose *C*-glycoside dabsyl ketohydrazone ($C_{29}H_{41},N_5O_{12}S$, calc. $[M + Na]^+ = 706.24$).

quantitatively converted into their corresponding phenylhydrazones. This was apparent from the MS ions 90 mass units larger than the parent compounds (Figure 2d,g). Indeed, we found that it was not necessary to isolate the *C*-glycoside ketones and that the ketohydrazones formed readily and quantitatively in a one-pot reaction with the corresponding unpurified aqueous reaction supernatants.

There are a variety of commercially available reagents based on hydrazine chemistry, and we, therefore, sought to establish the generality of their reaction with the *C*-glycoside ketones (Figure 3). As expected, a model compound, lactose *C*-glycoside ketone, reacted quantitatively with phenylhydrazine to give the corresponding phenylhydrazone (Figure 3c). Similarly, reaction with (carboxymethyl)pyridinium hydrazide or (carboxymethyl)-trimethylamino hydrazide (Girard's reagents P and T, respectively) quantitatively gave their corresponding lactose *C*-glycoside ketohydrazones (Figure 3d and Supporting Information). These derivatives have a fixed positive charge on the quaternary amino group, with the potential for enhanced sensitivity to positive ion detected mass spectrometry, and may also influence CID fragmentation patterns.[22−26] An analogous biotin-label lactose *C*-glycoside ketohydrazone was prepared from biotinyl hydrazine (Figure 3e). As discussed below, biotinylated carbohydrates have potential value for immobilization and affinity reagent by making use of biotin−avidin chemistry.[13,27] Reaction of a fluorescent reagent, dansyl hydrazine, with lactose *C*-glycoside ketone produced a fluorescent green ketohydrazone derivative (m/z 652.1, Figure 3f). The intensely red colored reagent, dabsyl hydrazine, gave rise to the corresponding lactose *C*-glycoside ketohydrazone, detected by MALDI-MS (m/z 706.3, Figure 3g). The dabsyl chromophore is based on the common pH indicator methyl orange (*p*-dimethylamino-azobenzene-sulfonic acid), and the carbohydrate derivatives based on this functionality were intensely red colored below pH 3.0. As demonstrated in an example below, we expect these derivatives to find application for the detection and chromatography of carbohydrates.

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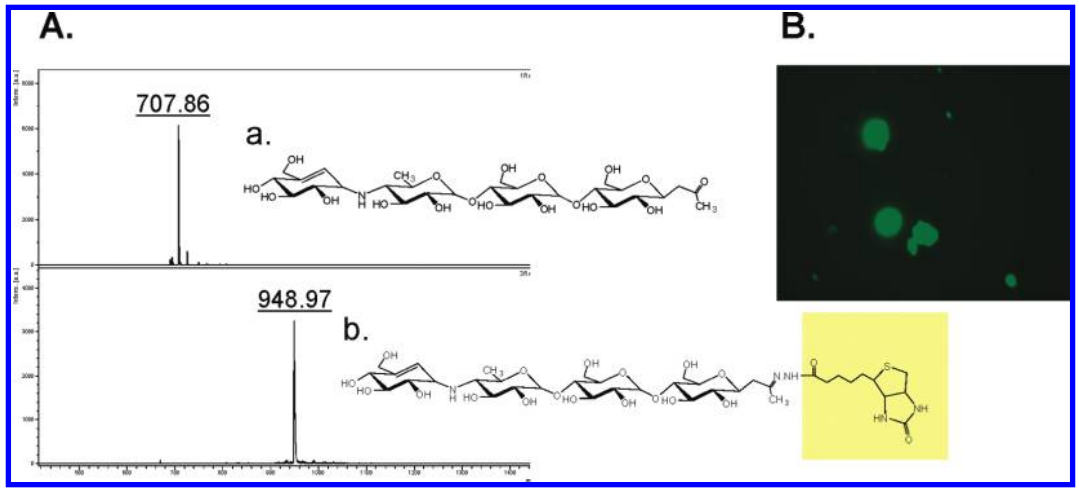

Figure 4. *C*-glycoside biotinyl ketohydrazone immobilization of acarbose, an antidiabetic oligosaccharide drug. (A) MALDI-TOF MS spectra of a, acarbose *C*-glycoside ketone; b, acarbose *C*-glycoside biotinyl ketohydrazone. (B) Streptavidin-functionalized beads treated with acarbose *C*-glycoside biotinyl ketohydazone and overlaid with fluorescent lectin, FITC-concanavalin A.

Applications to Carbohydrate Detection, Immobilization, and Enzyme Digestion of Biologically Important Oligosaccharides. To demonstrate the utility of our strategy, we present three applications. First, the potential for immobilization of oligosaccharide *C*-glycosides by biotin-streptavidin chemistry and its application to a clinically important antidiabetic drug, acarbose. As illustrated in Figure 4A, this drug was quantitatively converted in a one-pot reaction into its reducing-end activated biotin derivative that can then be immobilized onto streptavidin-functionalized beads (Figure 4B). Second, we show that a slightly modified approach (using biotin oximes) is applicable to naturally occurring hemicellulose oligosaccharides isolated from a plant cell wall xyloglucan (Supporting Information, Figure S.2.). Third, we show the potential of the red colored dabsyl *C*-glycoside ketohydrazones for monitoring the progress of cellulase enzymatic activity (Figure 5).

Because biotin−avidin affinity procedures are of increasing interest to glycobiology,[27] we first investigated the one-pot reaction of various oligosaccharide *C*-glycoside ketones with the ketone-reactive reagents, biotin hydrazine, and biotin hydroxylamine. Maltose oligosaccharides, either as mixtures or as discrete dp oligosaccharides (maltotriose, maltotetraose, maltoheptaose), were readily converted into *C*-glycoside ketones (Figure 2) and on treatment with commercial biotin hydrazine gave the corresponding biotin-labeled sugars (Figure 2 and Supporting Information). Acarbose is an α-glycosidase inhibitor and a tetrasaccharide analog of maltotetraose. It was quantitatively converted to its *C*-glycoside ketone (m/z 707.86) and, hence, to its biotinyl hydrazone (m/z 948.97) (Figure 4). As before, these reactions were achieved in aqueous, one-pot reactions, without the need of isolating the intermediate *C*-glycoside ketones. As an example application, we immobilized the acarbose biotin *C*-glycoside ketohydrazone onto streptavidin-functionalized beads, making use of the very high affinity (10^{15} M^{-1}) of avidin for biotin. Treatment with a dye-conjugated lectin (FITC-ConA) resulted in fluorescent labeling of the beads, as observed by fluorescent microscopy (Figure 4).

A second application was realized by treatment of a naturally occurring mixture of heptasaccharide (XXXG, m/z 1085.3), octasaccharide (XXLG, m/z 1247.4), and nonasaccharide (XLLG, m/z 1409.4) xyloglucans isolated from plant cell walls (Supporting Information, Figure S.2.). Aqueous reaction with acetylacetone afforded the corresponding *C*-glycoside ketones (m/z 1125.3, 1287.3, 1449.4, respectively; i.e., plus 40 Da) with good yield. Subsequent treatment with the ketone-reactive biotin hydroxylamine gave the biotin labeled xyloglucans (Supporting Information, Figure S.2.). Thus, diverse biotin-labeled carbohydrates are achievable by this methodology, permitting applications with lectins, carbohydrate−protein interactions, and immobilized sugars.

As a third example application of this novel carbohydrate "click chemistry", we prepared a colored substrate, celliobiose *C*-glycoside dabsyl ketohydrazone, for the evaluation of several commercial enzymes involved in cellulose degradation. This topic is presently of considerable interest for bioethanol production. The colored substrate and the expected enzyme product (glucose *C*-glycoside dabsyl ketohydrazone) were synthesized and characterized by MALDI-MS, LC-MS and TLC. The colored cellobiose derivative was observed by MALDI-MS to produce an $[M + Na]^+$ adduct ion at m/z 706, as seen previously for the dabsyl ketohydrazone of another disaccharide, lactose *C*-glycoside (Figure 3). Following treatment with cellulase, a new ion was observed at m/z 544, comparable to the mass of the glucose *C*-glycoside dabsyl ketohydrazone standard (Figure 5). A parallel evaluation of four cellulase preparations was undertaken by this method, highlighting the considerable difference in their ability to hydrolyze the terminal β-1,4-linked glucose residue (Figure 5). No activity was observed in the absence of cellulase enzymes. All four cellulases tested recognized the red colored cellobiose *C*-glycoside dabsyl ketohydrazone substrate and catalyzed the release of glucose as if from native cellobiose. This enzyme−substrate recognition, therefore, provides further evidence that the *C*-glycoside ketohydrazone derivatives retain the general conformation of the parent sugar.

DISCUSSION

Methods for the synthesis of *C*-glycosides are usually based on nucleophilic attack on a carbohydrate anomeric carbon, chemistry that generally requires activated nucleophiles and expensive protecting groups. The Knoevenagel reaction of unprotected aldose monosaccharides in aqueous conditions with

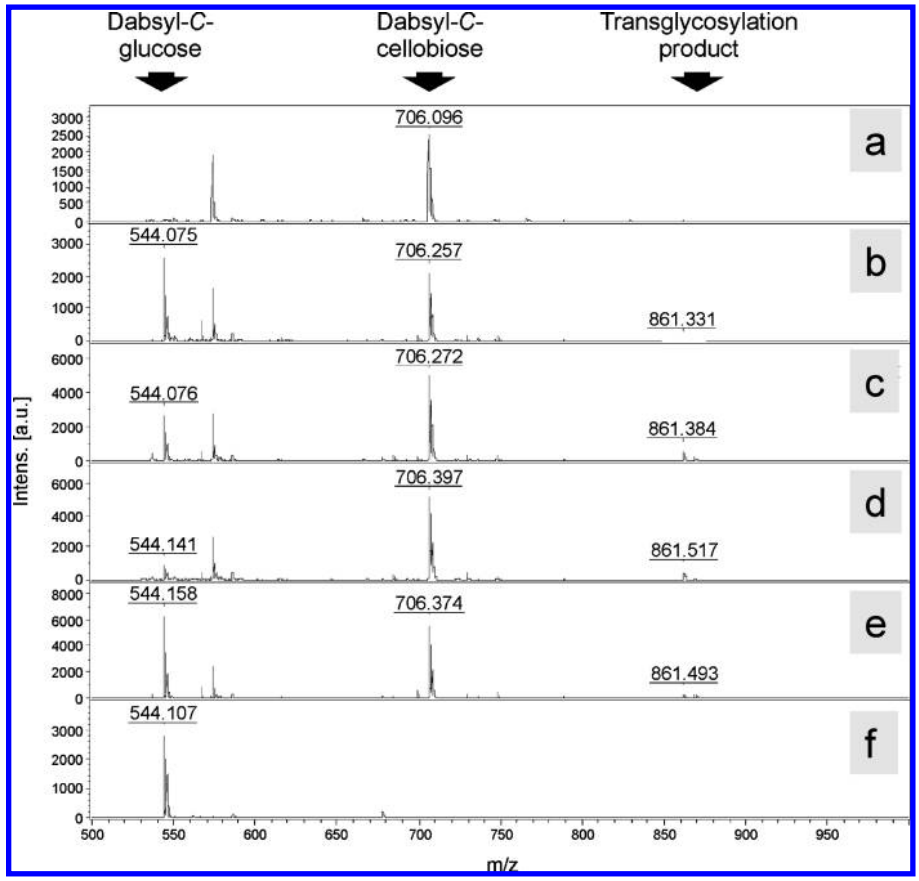

Figure 5. Enzymatic activity of commercial cellulases on cellobiose *C*-glycoside dabsyl ketohydrazone. The red colored derivative cellobiose *C*-glycoside dabsyl ketohydrazone (dabsyl-*C*-cellobiose, *m*/*z* 706) was hydrolyzed with various cellulase enzymes. The products, glucose *C*-glycoside dabsyl ketohydrazone (Dabsyl-C-glucose, *m*/*z* 544) and a glucotriose *C*-glycoside dabsyl ketohydrazone transglycosylation product (m/z 861), were analyzed by MALDI-TOF mass spectrometry. a, cellobiose *C*-glycoside dabsyl ketohydrazone untreated control; b, *Trichoderma viride* cellulase treatment; c, *Aspergillus niger* cellulase treatment; d, treatment with commercial cellulase from Cooper Biomedical; e, *Aspergillus niger* β-glucosidase treatment; f, glucose *C*-glycoside dabsyl ketohydrazone control.

β-diketones is known to be an efficient method of preparing *C*-glycoside ketones. This proceeds with high stereospecificity giving near quantitative yields. The simplicity of the reaction is such that it can be undertaken without specialized knowledge or facilities for carbohydrate synthesis. Using MALDI-MS to monitor the reaction, we showed that the reaction is equally applicable to a variety of oligosaccharides of varying degree of polymerization and structure. Indeed the only structural requirement appears to be for the presence of an aldose residue at the carbohydrate reducing terminus with an unsubstituted anomeric position. Hence, the reaction with acetylacetone was generally applicable to hexose- and pentose-containing oligosaccharides. Furthermore, the reaction conditions (pH 8.5, 80 °C) are mild enough that no degradation of the oligosaccharide chain occurred. Other substituents such as *N*-acetyl and *O*-acetyl groups were also unaffected (see Supporting Information).

Another advantage of the *C*-glycoside ketones is that the derivatized sugar residue retains the ring integrity of the parent sugar. Analysis of MS fragmentation pathways and preliminary conformational analysis by NMR indicate that the hexose *C*-glycoside ketones are predominantly $^4C_1$ cyclic chair structures. It, therefore, seems likely that small disaccharide derivatives, such as those of cellobiose and lactose, are structurally conserved at the reducing terminus, and for this reason, cellobiose *C*-glycoside dabsyl ketohydrazone is recognized as an enzyme-specific cellulase substrate. There is also further scope for the "locked ring" aspect of the *C*-glycoside ketohydrazone methodology reported, particularly in relation to glycoarray preparation. For example, the hydrazone array work of Shin concludes that the stronger binding of proteins to carbohydrates immobilized on hydrazide-coated beads than their oxime-linked counterparts is a consequence of the higher ratio of cyclic to acyclic linkages obtained with the former.[28] A similar carbohydrate hydrazide approach developed by Zhi et al. for gold surfaces[29] might also have improved affinity and, hence, improved sensitivity, by the use of *C*-glycoside ketones rather than unmodified sugars. Hence, the methods described here to synthesize *C*-glycoside ketohydrazone glycoconjugates will enable a wide variety of biophysical tools to be used to study carbohydrate−protein interactions.

The general applicability of the reaction and the introduction of the exoketone group, suggested an immediate way in which the *C*-glycoside ketones could be used in a general strategy to label or immobilize sugars using commercially available ketone-reactive reagents. Initially, reactions with simple hydroxylamines and hydrazines indicated that ketooximes and ketohydrazones

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formed readily under aqueous conditions, and this could be applied directly to the *C*-glycoside ketone intermediates in one-pot reactions without prior isolation. Hydrazone and oxime formation was observed to be essentially quantitative, and the remote exoketone group also overcame the potential problems of sugar osazone formation.

There are a large number of ketone-reactive reagents available for labeling or immobilization via carbonyls, including fluorescent and colored hydrazines and hydroxylamines, biotin labels, functionalized resins, and cationic groups such as the Girard's reagents. Moreover, the simplicity for sulfonyl- and acyl-hydrazine formation should extend these applications.[30] As examples, we have described several oligosaccharide *C*-glycoside ketohydrazones labeled with the fluorescent dansyl (dimethylaminonaphalene sulfonyl) group, the colored dabsyl group, Girard's labels suitable for sensitive positive ion detected mass spectrometry, and the avidin-responsive biotin label. These chemistries were also applicable to naturally occurring carbohydrate mixtures, such as xyloglucans, and acarbose, a commercially important antidiabetic analog of maltotetraose. Applications for the labeled carbohydrates include lectin and protein binding studies, as readily detectable substrates for carbohydrate-processing enzymes. As an example, we have shown the immobilization of biotin-labeled acarbose *C*-ketohydrazone to streptavidin beads and its subsequent binding to a fluorescently labeled lectin. This type of visual assessment can often be modified for use in high throughput screening of potential binding inhibitors. Finally, an application of red-colored dabsyl-labeled cellobiose *C*-glycoside ketone has been presented for the screening of cellulase activity. These advances could allow for simple testing of multiple glycosylase activities and represent potent and generally applicable new tools for glycomics.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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